Phytoprotection

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Volume 86, numéro 1, avril 2005

URI : https://id.erudit.org/iderudit/011712ar
DOI : https://doi.org/10.7202/011712ar

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Éditeur(s)

Société de protection des plantes du Québec (SPPQ)

ISSN

0031-9511 (imprimé)
1710-1603 (numérique)

Découvrir la revue

Citer cet article

Riga, E., Hooper, C. & Potter, J. (2005). *In vitro* effect of marigold seed exudates on plant parasitic nematodes. *Phytoprotection*, *86*(1), 31–35. https://doi.org/10.7202/011712ar

Résumé de l'article

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In vitro effect of marigold seed exudates on plant parasitic nematodes

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Received 2004-10-12; accepted 2005-04-05

PHYTOPROTECTION 86 : 31-35

Water extracts from seed exudates of *Tagetes erecta* cv. Crackerjack and *T. patula* var. *polynema* caused significantly higher mortality ($P < 0.05$) to *Heterodera schachtii*, *Meloidogyne hapla* and *Pratylenchus penetrans* than the control extracts from radish, tomato and corn seeds, respectively. Marigold seed exudates consist of nematicidal compounds. Nematostatic compounds have not been found in the seed exudates. Two high-performance liquid chromatography fractions derived from *T. erecta* cv. Crackerjack and one from *T. patula* var. *polynema* caused the highest mortality of *H. schachtii* in comparison with a water control and the rest of the fractions.

Keywords: Nematicidal compounds, *Tagetes erecta*, *Tagetes patula*.

[Effet *in vitro* d'extraits liquides de graines de soucis sur des nématodes phytopathogènes]

La mortalité de *Heterodera schachtii*, de *Meloidogyne hapla*, et de *Pratylenchus penetrans* causée par des extraits liquides obtenus à partir des graines de *Tagetes erecta* cv. Crackerjack et de *T. patula* var. *polynema* était significativement ($P < 0,05$) plus grande que chez les témoins. Les extraits de graines contiennent des produits avec un effet nématicide. Des produits nématostatiques n'ont pas été trouvés dans les extraits. Deux fractions de chromatographie liquide à haute performance dérivées de *T. erecta* cv. Crackerjack et une fraction dérivée de *T. patula* var. *polynema* ont causé la plus grande mortalité de *H. schachtii* en comparaison des témoins et du reste des fractions.

Mots clés : *Tagetes erecta*, *Tagetes patula*, composés nématicides.

Plant-parasitic nematodes are cosmopolitan parasites of many crops worldwide and are responsible for losses of 10 to 50% within a single crop yield (Barker *et al.* 1998). The sugar beet cyst nematode, *Heterodera schachtii* Schmidt, occurs in many parts of the world including Canada and the US (Baldwin and Mundo-Ocampo 1991). This nematode infects numerous cruciferous plants including sugar beet, *Beta vulgaris* L. Up to 90% of damage to sugar beets has been reported as a result of infection with *H. schachtii* (Steele 1984). The root-knot nematode, *Meloidogyne hapla* Chitwood, and the lesion nematode, *Pratylenchus penetrans* Cobb, occur in temperate regions, infect a wide range of plants worldwide, and cause varying degrees of economic losses (Barker *et al.* 1998; Loof 1991). Until recently, control of these nematodes was achieved by synthetic nematicides, some of which are environmentally undesirable (Dunn and Noling 1997). Recently, the need for novel plant-derived nematicides that are non-persistent, biodegradable and non-toxic to non-target organisms has been increasing.

Marigold, *Tagetes* spp., has been known to produce nematicidal compounds (Kimpinski *et al.* 2000; Ploeg 1999; Sipes and Arakaki 1997). One of the main marigold compounds with nematicidal properties is a thiophene photoactivated compound called $\propto$-terthienyl (Bakker *et al.* 1979; Hasan 1992).

The effect of marigold seed exudates on nematodes has not been investigated. The purpose of this project was to investigate the nematicidal properties of marigold seed exudates and to identify potential sources that will lead to novel nematicidal plant-derived compounds.

Seeds of *T. patula* L. (var. *polynema*) and *T. erecta* L. (cv. Crackerjack) (Stokes Seeds Ltd., St. Catharines, ON, Canada) (0.2 g each) were washed and wrapped in a nylon-permeable filter and placed in individual 6-cm diam Petri dishes filled with 10 mL dechlorinated tap water for 72 h at 25°C. Seeds of radish, *Raphanus sativus* L., corn, *Zea mays* L., and tomato, *Lycopersicon esculentum* Mill., were used as controls for *H. schachtii*, *P. penetrans* and *M. hapla*, respectively. At the end of the incubation period, 20 juveniles of each of *H. schachtii*, *P. penetrans* and *M. hapla*

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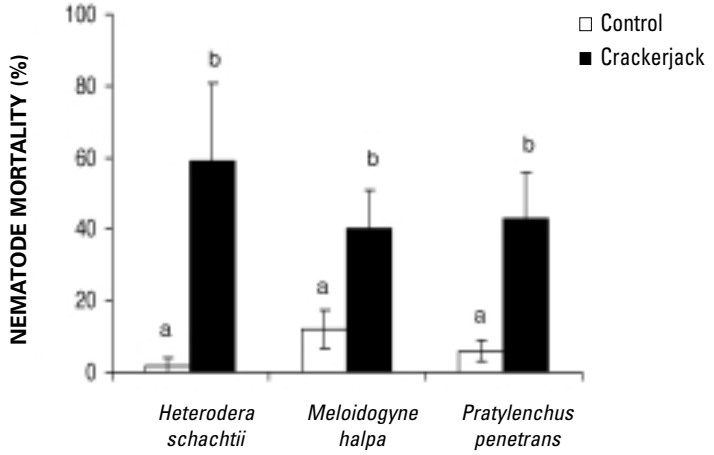

Figure 1a. The effect of water extract from seed exudates of *Tagetes erecta* (cv. Crackerjack), and radish, corn, and tomato controls on three species of plant-parasitic nematodes. Data for each column are averages ± standard deviations of 30 replications. The same letter between treatments and control indicates no difference at $P < 0.05$.

were added to each marigold seed exudates and control and incubated for 48 h. Nematode mortality rates were assessed using a compound microscope after 48 h. Nematodes that did not respond to physical probing using a metal probe were considered either dead or paralyzed. To determine whether marigold seed exudates were nematicidal (i.e. nematodes are dead) or nematostatic (i.e. nematodes are temporarily paralyzed), 20 nematodes that were incubated for 48 h in exudates were transferred to 5 mL dechlorinated tap water at 25°C for 24 h. The nematodes were then observed under a compound microscope for signs of recovery and nematode mortality. Ten Petri dishes were used per exudate from either marigold or control for each nematode species. All assays were repeated three times.

Exudates from marigold seeds that were incubated in dechlorinated tap water for 72 h were collected, filtered using filter paper (#5 qualitative filter paper, Whatman International Ltd., England) and placed into 200-mL scintillation vials. Each set of exudates was analyzed using a Hewlett Packard 1090 high-performance liquid chromatography (HPLC) apparatus with a U.V. detector (Hewlett-Packard, Waldbronn, Germany), and a DuPont Zorbax PSM 60 6.22X 250 column (Wilmington, DE, USA) at 40°C. The solvents used

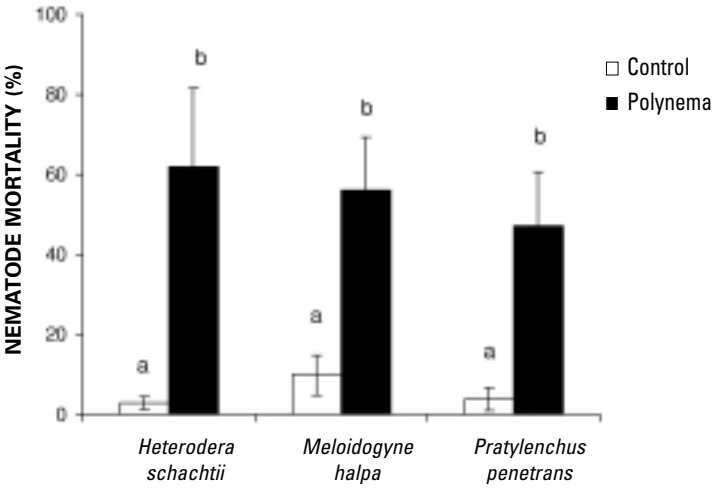

Figure 1b. The effect of water extract from seed exudates of *T. patula* (var. *polynema*), and radish, corn, and tomato controls on three species of plant-parasitic nematodes. Data for each column are averages ± standard deviations of 30 replications. The same letter between treatments and control indicates no difference at $P < 0.05$.

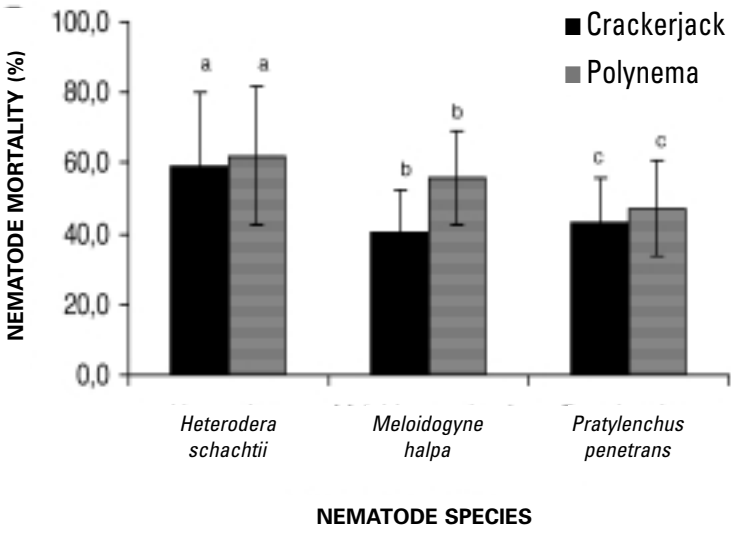

Figure 1c. The effect of water extract from seed exudates of *T. patula* (var. *polynema*) and *T. erecta* (cv. Crackerjack) on three species of plant-parasitic nematodes. Data for each column are averages ± standard deviations of 30 replications. The same letter between treatments of the same species indicates no difference at $P < 0.05$.

were acetonitrile and tap water that had been prepared using millipore filtration. Exudates of each marigold variety (250 μL) were injected into the column at a flow rate of 1 mL min^{-1} and each HPLC run lasted for 20 min. Four runs per sample were performed. Dechlorinated tap water was used as control. The derived fractions were collected and 250 μL of each fraction was added to a microcavity slide (16 mm x 3 mm) (Clay Adams, New York, USA) with 20 *H. schachtii* juveniles. The slides were sealed to prevent evaporation and incubated for 72 h at 25°C, after which recovery and nematode mortality rates were assessed. For control treatment, nematodes were put in a slide containing water. Due to the limited HPLC fraction quantities extracted from marigold seeds, only *H. schachtii* was tested in the consecutive assays since this nematode appeared more sensitive to the effects of marigold seed exudates. HPLC fractionation of marigold seed extracts and nematode assays were repeated three times. Data from the three repetitions were combined. Prior to statistical analysis, the combined data were tested for homogeneity of variances (Devore 1987). Statistical differences ($P < 0.05$) were tested using the Kruskal-Wallis test and Tukey's multiple comparisons method (Devore 1987) among exudates for each nematode in the mortality assays and among the HPLC fractions for each marigold species.

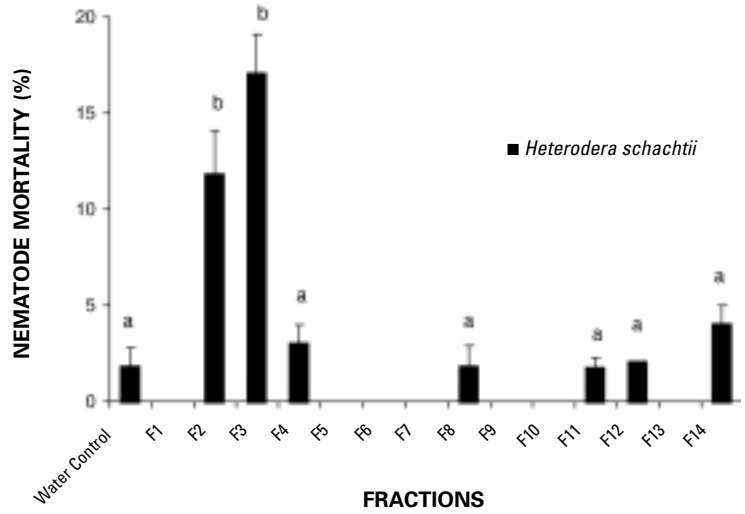

Figure 2. The effect of HPLC fractions derived from water extract from seed exudates of *Tagetes erecta* (cv. Crackerjack) and water control on *Heterodera schachtii*. Data for each column are averages ± standard deviations of 3 replications. The same letter between treatments indicates no difference at $P < 0.05$.

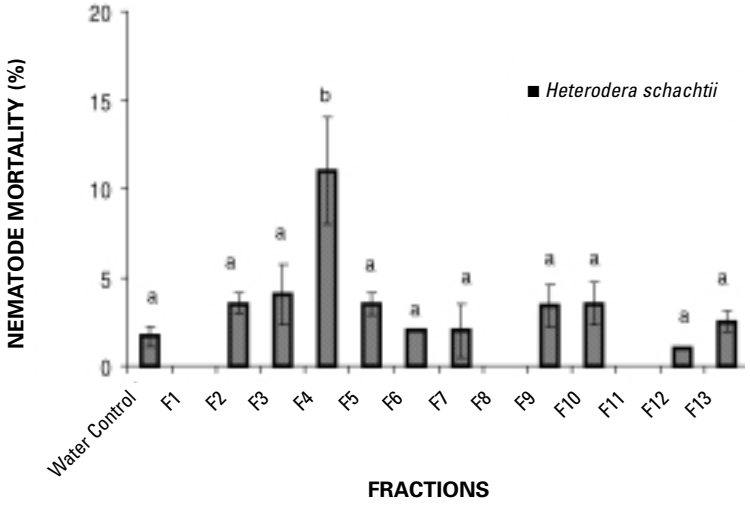

Figure 3. The effect of HPLC fractions derived from water extract from seed exudates of *Tagetes patula* (var. *polynema*) and water control on *Heterodera schachtii*. Data for each column are averages ± standard deviations of 3 replications. The same letter between treatments indicates no difference at $P < 0.05$.

Seed exudates of *T. erecta* (cv. Crackerjack) and *T. patula* (var. *polynema*) caused significantly higher mortality to *H. schachtii*, *M. hapla* and *P. penetrans* than did their respective controls ($P < 0.05$) (Fig. 1a and 1b). There was no significant difference between the mortality rates caused by the two marigold varieties. In addition, there was no significant difference between the mortality rates caused by each of the marigold seed extracts in each individual nematode species (Fig. 1c), although the trend suggests that *T. patula* (var. *polynema*) caused the highest mortality to *H. schachtii*. No significant nematode recovery was observed in comparison with the controls when the nematodes were transferred from the marigold seed exudates to water for 24 h (data not shown).

The HPLC fractions F2 and F3, derived from *T. erecta*, caused the highest mortality to *H. schachtii* juveniles in comparison with the water control and the rest of the fractions (Fig. 2). HPLC fraction F4, derived from *T. patula*, caused significantly ($P < 0.05$) higher mortality to *H. schachtii* juveniles than the control and the rest of the HPLC fractions (Fig. 3). No nematode recovery was observed in comparison with the control when nematodes were transferred from HPLC fractions to water for 24 h (data not shown).

Seed exudates from both *Tagetes erecta* (cv. Crackerjack) and *T. patula* (var. *polynema*) caused significantly higher mortality to *H. schachtii*, *M. hapla* and *P. penetrans* compared with control exudates from radish, corn and tomato seeds. Several reports exist on the nematicidal properties of marigolds from different parts of the plant (Alexander and Waldenmaier 2002; Kimpinski *et al.* 2000; Ploeg 1999; Siddiqui and Alam 1988). However, the nematicidal properties of marigold seed exudates in the laboratory or field have not been thoroughly investigated. Two HPLC fractions derived from *T. erecta* cv. Crackerjack and one fraction from *T. patula* var. *polynema* caused the highest *H. schachtii* mortality in comparison with the water control and the rest of the HPLC fractions. Our study shows that seed exudates contain nematicidal compounds. No nematostatic compounds were found in these exudates since nematode recovery was not observed.

Additional work is needed to isolate and characterize compounds derived from marigold seed exudate fractions. Identifying new compounds may potentially lead to the discovery of novel plant-derived nematicides.

ACKNOWLEDGEMENTS

The authors sincerely thank Dr. Richard Larsen, USDA-ARS, WA, for reviewing the manuscript.

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